Drafted 03/08/82
Revised 10/02/85
Revised 06/11/86
Revised 08/13/86
Revised 09/12/90
Revised 09/30/92
Revised 09/13/95
Reapproved 05/08/96
Revised 04/09/97
Revised 03/27/02

Guidelines for NIH Intramural Research Program Compliance with USDA Annual Reporting Requirements

Animal Welfare Regulations (AWRs) require each reporting facility to submit an annual report to the Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) on or before December 1 of each calendar year. All Institute/Center (IC) programs must submit this report (APHIS Form 7023) to the Office of Animal Care and Use (OACU) each November. The OACU compiles the IC reports into one NIH report. A sample APHIS Form 7023, Form 7023A (Additional), and a sample form titled "Explanation for Column E Listing" are appended as Attachments 1, 1A, and 1B respectively. Please read the sample Form 7023 and carefully follow the form's instructions, including the typed-in description of information that is needed for completing the form. Some areas on the form must be left blank as indicated on the sample.

The Scientific Directors of all IC's that use animals must sign the form as the Institutional Official within that IC. Their signature assures the USDA that the IC is in compliance with the four assurances stated near the bottom of the first page of the form.

GENERAL GUIDELINES

The intent of these guidelines is to standardize the compilation and reporting of animal use in the NIH annual report to the USDA. The objectives of these instructions are to 1) clarify the word "used" in the context of the annual report and 2) provide assistance in selecting the correct columns on Form 7023 for recording the numbers of animals used; examples are included.

Only vertebrate species are reported in the annual report. In this document, the words "use" or "used" refer to the incorporation of vertebrate animals in teaching, testing, experiments, or research. Animals must be reported each year they are used.

In this document, instructions concerning Columns B, C, D and E are not applicable to rats of the genus Rattus or mice of the genus Mus. Rats and mice of any other genus are covered by the AWRs and must be listed appropriately in Columns A through F.

Regulated species being bred, conditioned, or held for use, but not yet used as of September 30 are listed in Column B of the report. The numbers listed in this column reflect a one-day inventory (usually performed September 30 of the reporting period) of

animals being bred, conditioned, or held for use, but not yet used during the reporting year.

All animals of regulated species which have been used during the reporting period are listed in Column C, D, or E of the annual report. Sentinel animals (other than rats of the genus Rattus and mice of the genus Mus) are considered to have been used, and should be counted and entered in the appropriate column.

Offspring of regulated species are reported in Column C, D, or E if they have been used on an Animal Study Proposal (ASP) or in Column B if they have been weaned but not used and are present as of September 30 of the reporting year. If a pregnant animal of a regulated species is administered a substance to study its effect on the offspring (i.e. the offspring are used to generate data), the offspring and their mother are listed in the appropriate column(s) of the annual report (Columns C, D or E). When fetuses are collected, only the pregnant female is reported as having been used.

Reparative surgery or medical treatments provided or prescribed by a veterinarian for an animal due to illness or injury is considered normal veterinary care and **does not** determine the placement of the animal in Columns C, D or E. Likewise, routine veterinary procedures such as castrations, dehorning and diagnostic procedures performed or prescribed by a veterinarian are considered normal veterinary care and do not determine the placement of the animals in Columns C, D or E. Veterinary interventions to treat or correct medical/surgical conditions resulting from research procedures **are** used to determine the placement of an animal in Columns C, D or E.

Animals that were assigned to more than one ASP by an IC during the reporting period are reported **only once for that year**, but should be listed in the columns (C, D or E) consistent with the greatest amount of accompanying pain or distress they were subjected to during the reporting period.

COLUMN A

Column A of the form contains a preprinted partial list of regulated species by **COMMON NAMES** (e.g. dogs, cats, etc.) and space to enter the common names of other regulated species. At the NIH, the common names of all vertebrate animal species being held, bred, or used must be listed in Column A.

COLUMN B

Column B listings represent the number of animals being bred, conditioned, or held for future use. The numbers listed in this column reflect a one-day inventory (usually September 30 of the reporting period) of animals being bred, conditioned, or held for use, but not yet used for such purposes during the reporting year. This is not a tally of

the animals held for use throughout the year, but a tally of animals not yet used as of September 30 of the reporting year.

EXAMPLE B1: A breeding colony of squirrels was established to provide animals for research. The animals were not subjected to any procedures except breeding, rearing of offspring, and holding for future use. On September 30, the mated pairs (animals being bred) and their weaned offspring (animals being held for future use), are counted and reported in Column B.

EXAMPLE B2: As of September 30 of the reporting period, a breeding colony of 10 hamsters had 94 weaned offspring and 27 offspring that were still nursing. None of the hamsters had been used. All of the adult hamsters and their weaned offspring are listed in Column B. The 27 offspring still nursing and not yet used to generate data for an ASP are not listed on the annual report.

EXAMPLE B3: Twenty male and 20 female cotton rats were bred and 165 offspring were weaned during the reporting period. Forty five of the offspring and the 40 breeding cotton rats were not subjected to any experimental procedures during the fiscal year and are listed in Column B. One hundred of the offspring were used in research procedures during the reporting period and are listed in the appropriate USDA column (Columns C, D, or E - see below). Twenty of the offspring were euthanized prior to September 30 of the reporting period with no experimental procedures being performed on them. These twenty cotton rats are not listed on the annual report.

COLUMN C

The animals listed in this column were not subjected to procedures that involved pain and/or distress or to the use of anesthetics, analgesics or tranquilizers to prevent pain and/or distress. The AWRs state that routine procedures producing no or only minimal or transient pain should be reported in this column (see below). The numbers reflect a **retrospective summation** of animals on ASPs that were not subjected to pain or distress. Animals promptly euthanized when morbidity is first observed are also listed in Column C.

<u>TYPES OF PROCEDURES LISTED IN COLUMN C.</u>

The following procedures are examples of Column C procedures when performed by trained individuals. The list is not meant to be definitive:

1. Administration of:

    a. Electrolytes and other fluids

b. Immunizations, including the proper use of Complete Freund's Adjuvant (CFA) - see Animal Research Advisory Committee (ARAC) "Recommendations for Consideration in the Research Use of Inflammatory Agents"

c. Oral medications.

2. Most blood collection procedures.

3. Gastric gavage.

4. The administration of an anesthetic, analgesic or tranquilizing drug to an animal for short term restraint purposes to perform a procedure that involves no pain or distress.

5. Non-surgical catheterization.

6. Certain manipulative procedures such as injections, palpations, skin scrapings, and radiography.

7. Intracerebral inoculations in neonatal rodents prior to cranial ossification when performed by trained personnel.

8. Euthanasia performed in accordance with the recommendations of the most recent Report of the AVMA Panel on Euthanasia, utilizing procedures that produce rapid unconsciousness and subsequent humane death with minimal or no pain or distress, such as the use of inhalant anesthetics, carbon dioxide, parenterally administered barbiturates, cervical dislocation, and decapitation, the latter two requiring justification and Animal Care and Use Committee (ACUC) approval.

9. Chair restraint of an adapted nonhuman primate (NHP) that has been conditioned for the time period of restraint up to 12 hours, or the training of an unadapted NHP to chairing utilizing an ACUC approved plan that results in minimal or transient distress.

If the result of any of the above procedures is observed to be painful or distressful to the animals, the ACUC will be informed, the ASP modified or halted, and the animals listed in Column E.

EXAMPLE C1: Two hundred weaned hamsters received a drug subcutaneously that produced only minimal and transient pain. Two weeks later they were euthanized by CO_2 inhalation. These hamsters are listed in Column C.

EXAMPLE C2: Two rabbits were immunized with a mixture of an antigen and Complete Freund's Adjuvant following the ARAC "Recommendations for Consideration in the Research Use of Inflammatory Agents." No inflammatory lesions or tissue necrosis were observed at the site(s) of immunization during the reporting period and pain was not evident. These two rabbits are listed in Column C.

EXAMPLE C3: Twenty euthymic female guinea pigs were inoculated intravaginally with the Herpes simplex virus. The guinea pigs were examined twice-a-day for genital lesions and euthanized immediately when herpetic vesicles were observed. These guinea pigs are listed in Column C.

EXAMPLE C4: A macaque was anesthetized with ketamine and an appropriate volume of blood was obtained from the femoral vein for an investigator. This animal is listed in Column C.

EXAMPLE C5: An appropriate volume of an isotonic solution was instilled into the upper tracheas of three dogs and promptly removed. The dogs exhibited no signs of pain and only minimal distress from the procedure. List these dogs in Column C.

EXAMPLE C6: Five hamsters are inoculated intraperitoneally with a hybridoma cell line and the ascitic fluid is removed from the peritoneal cavity three times. All hamsters are alert, active, and eating and drinking normally. The hamsters are euthanized and the remaining ascitic fluid removed postmortem. These hamsters are listed in Column C.

COLUMN D

Animals that are used in procedures which would involve more than minimal or transient accompanying pain or distress, but for which appropriate and adequate anesthetic, analgesic, tranquilizing drugs, or other procedures performed to ensure no more than minimal or transient pain or distress, must be listed in Column D. Listings in this column represent a **retrospective summation** of animals that were used during the year under the conditions described in the heading of Column D.

TYPES OF PROCEDURES LISTED IN COLUMN D.

Examples of procedures that may produce pain or distress as defined in Attachment 2, but which are performed using anesthetics, analgesics or tranquilizers appropriate to prevent or alleviate pain or distress are:

1. Surgery, including biopsy, gonadectomy, neurophysiological manipulations or preparations such as the implantation of electrodes and recording devices.

2. Terminal surgical procedures in which the animal(s) are euthanized before recovering from anesthesia.

3. Periorbital collection of blood in species without a true orbital sinus, such as rats and guinea pigs.

4. Intracardiac blood collection.

5. Euthanasia performed in accordance with the recommendations of the most recent Report of the AVMA Panel on Euthanasia utilizing procedures that would involve pain or distress if anesthetics, analgesics or tranquilizer drugs were not used, such as pithing in certain species and exsanguination.

EXAMPLE D1: Twenty dogs were assigned to an ASP involving a major survival surgical procedure, but two were actually used as nonsurgical controls and experienced no pain or distress. List those two animals under Column C and the other 18 under Column D.

EXAMPLE D2: Five cats were deeply anesthetized with pentobarbital and perfused with formalin. These animals are listed under Column D.

COLUMN E

Animals must be listed in Column E if they are subjected to procedures involving more than minimal or transient accompanying pain or distress in which appropriate anesthetics, analgesics, or tranquilizing drugs could not be used because their use would have adversely affected the teaching, testing, experiments, or research or in which the use of anesthetics, analgesics, tranquilizing drugs, or other procedures performed to prevent pain or distress were not adequate to preclude more than minimal and transient pain or distress.

Retrospectively, list only those animals that were used and experienced pain or distress during the reporting year, rather than listing the number of animals that were approved in the ASP. All animals approved for use in painful or distressful procedures without appropriate and adequate anesthetics, analgesics or tranquilizers may not have experienced pain or distress. Animals assigned to the ASP, but not actually used in Column E conditions, are listed in the appropriate column (Column B, C or D).

If regulated species are used under Column E conditions, the Column E justification form (Attachment 1B) must be attached to the annual report and a copy appended as an attachment to the ASP. The Column E justification forms **must be signed and dated at the end of the reporting period.**

TYPES OF PROCEDURES LISTED UNDER COLUMN E.

Examples of procedures that must be listed in Column E when performed without the use of anesthetics, analgesics or tranquilizing drugs include:

1. The chairing of a NHP which has not been conditioned for the time period of restraint.

2. Drug or radiation toxicity testing producing unrelieved pain and/or distress.

3. LD_{50} determinations.

4. The exposure of an animal to an agent which produces unrelieved pain or distress.

5. The exposure of an animal to electrical shocks that are generally accepted as causing pain in humans.

EXAMPLE: Twenty nonhuman primates (NHP) were inoculated intravenously with a splenic-derived cell suspension infected with the simian immunodeficiency virus. During the reporting period, two of the NHPs had debilitating chronic diarrhea with weight loss and/or dehydration and one NHP had clinically evident pneumonia as a result of their infection with SIV in spite of appropriate veterinary support. These three animals must be listed in Column E because they experienced unrelieved pain or distress during the reporting period.

COLUMN F.

Column F listings are the sum of the animals listed in Columns C, D, and E, by species.

COMMENTS AND QUESTIONS RELATING TO THESE GUIDELINES:

Address comments or questions to the NIH Animal Research Advisory Committee through the Office of Animal Care and Use. Telephone 301-496-5424 or FAX 301-480-8298.

Attachment 1B

Column E Explanation Form

This form is intended as an aid to completing the Column E explanation. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

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1. **Registration Number: 51-F-0016**

2. **Number of animals used under Column E conditions in this study. _____**

3. **Species (common name) of animals used in this study. _____**

4. **Explain the procedure producing pain and/or distress, including reason(s) for species selected.**

5. **Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.**

Information below will NOT be forwarded to USDA as part of the Annual Report

IC_____ ASP Number_____ ASP Title_____

Signature of Principal Investigator_____Date _____

Attachment 2

DEFINITIONS AND BEHAVIORAL/CLINICAL SIGNS OF PAIN

PAIN *

Pain, as defined by the International Association for the Study of Pain is "an unpleasant sensory or emotional experience associated with actual or potential tissue damage". Pain can be considered a potent source of stress, that is a stressor. It can also be considered a state of stress itself and lead to distress and maladaptive behaviors. Thus, whether pain is considered as a kind of stress or as a stressor depends on the point of reference.

Acute Pain is abrupt in onset and relatively short in duration. It can result from an inflammatory process that originated in damaged tissue, traumatic injury, surgery, or exposure to metabolic, bacterial, or viral disease or toxins. Such pain produces a stress response, but usually does not lead to distress, because the pain is short-lived. The pain is generally alleviated by analgesics and associated distress may be responsive to tranquilizers.

*Chronic (*Persistent) *Pain* is slow in onset, its intensity is likely not constant, and it is not necessarily associated with an obvious pathologic condition. It is more likely to lead to distress and maladaptive behavior. Chronic pain generally is not totally alleviated by analgesics but associated distress may be alleviated by tranquilizers.

DISTRESS

"Distress is an aversive state in which an animal is unable to adapt completely to stressors and the resulting stress and shows maladaptive behavior."
The origin of these stressors can be categorized generally as physiologic, psychologic, or environmental.

Potential causes for physiologic stress are pain (resulting from injury, surgery, or disease), starvation, and dehydration. Potential causes for psychologic stress are fear, anxiety, boredom, loneliness, and separation. Environmental causes of stress include restraint, noise, odors, habitat, people, chemicals, and other animals. The appropriate intervention for alleviating distress depends on an accurate identification of the stressor(s) causing the distress.

* National Research Council (NRC). 1992. Recognition and Alleviation of Pain and Distress in Laboratory Animals. A report of the Institute of Laboratory Animal Resources Committee on Pain and Distress in Laboratory Animals, National Research Council, National Academy of Sciences. Washington, D.C.: National Academy Press. 137pp.